

**Amendments to the Specification:**

**Please replace the paragraph starting on line 11 of page 1 of the specification with the following amended paragraph:**

This invention relates to libraries of affinity markers, and especially F/YEE (SEQ ID NO:1), for human serum albumin. This invention is further related to methods of screening the affinity marker libraries, identification of specific markers and the use of the markers as delivery agents for therapeutic *in vitro* and *in vivo* diagnostic agents.

**Please replace the paragraph starting on line 9 of page 2 of the specification with the following amended paragraph:**

The present invention addresses these and other problems of the prior art by providing compounds having the formula E-C<sub>a</sub>-R-C<sub>b</sub>-A. In this formula, E contains an active diagnostic moiety or pharmacophore and suitable connectors, R is a potentially reactive functional group contained within the entity and is capable of transferring an active diagnostic moiety or pharmacophore. C<sub>a</sub> and C<sub>b</sub> are connector groups between E and R and between R and A, respectively. A is any molecule or part of a molecule that possess specific binding determinants for a target molecule, such as proteins including human serum albumin. (These components of compounds according to the present invention are defined more fully below.) In some compounds according to the invention, affinity group A comprises a sequence of amino acid residues -O<sub>1</sub>-O<sub>2</sub>-X<sub>1</sub>-X<sub>2</sub>-B in which the amino acid residues are independently selected from the group of all twenty naturally occurring amino acids. A particularly preferred sequence is F/YEE (SEQ ID NO:1). Preferred compounds according to the invention include biotin-S-Ph-C(O)-F/YEE-NH<sub>2</sub>, biotin-OPh-C(O)-F/YEE-NH<sub>2</sub>, LC-biotin-S-Ph-C(O)-F/YEE-NH<sub>2</sub>, biotin-Gly-OPh-C(O)-F/YEE-NH<sub>2</sub>, fluorescein-Gly-OPh-F/YEE-NH<sub>2</sub>, LC-biotin-OPh-C(O)-F/YEE-NH<sub>2</sub>, argatroban-AEA<sub>3</sub>-βAla-Gly-OPh-C(O)-F/YEE-NH<sub>2</sub> and fluorescein-thiourea-AEA<sub>3</sub>-Gly-OPh-C(O)-F/YEE-NH<sub>2</sub>, where LC is (for "long chain") has the formula -NH-(CH<sub>2</sub>)<sub>n</sub>-C(O)-, where n is an integer between 1 and 25. These compounds can bind to specific

proteins in vivo and enhance the half lives of diagnostic and therapeutic agents as well as control side effects.

**Please replace the paragraph starting on line 12 of page 3 of the specification with the following amended paragraph:**

Figure 1 shows the structural formula of the most preferred affinity group of the invention, -F/YEE (SEQ ID NO:1).

**Please replace the paragraph starting on line 26 of page 3 of the specification with the following amended paragraph:**

Figure 7 shows an immunoblot in which human serum and plasma samples were labeled with two different concentrations of biotin-OPh-CO- NH<sub>2</sub> to assess compound lack of specificity in the absence of F/YEE peptide (having SEQ ID NO:1).

**Please replace the paragraph starting on line 12 of page 9 of the specification with the following amended paragraph:**

The preferred amino acids at the X<sub>1</sub>, X<sub>2</sub>, and B positions are tyrosine, glutamic acid and glutamic acid respectively. The most preferred affinity group is F/YEE (SEQ ID NO:1), where F is phenylalanine, I is D-leucine, Y is tyrosine, and E is glutamic acid.

**Please replace the paragraph starting on line 15 of page 9 of the specification with the following amended paragraph:**

In a preferred embodiment, the B position amino acid residue is the C-terminal amino acid. In another preferred embodiment, the B position amino acid residue exists in its carboxamide form rather than in the carboxylic acid form. In this preferred embodiment the -F/YEE affinity group (SEQ ID NO:1) has the structural formula shown in Figure 1.

**Please replace the paragraph starting on line 22 of page 12 of the specification with the following amended paragraph:**

Figure 2 shows the reaction scheme for the use of a preferred embodiment of the present invention **5** to attach a biotin group **10** (the entity) to HSA **40** (the target molecule). In the preferred embodiment shown in Figure 2, the reactive group **20** is an -O-Ph-C(O)- group and the affinity group **30** is F/YEE (SEQ ID NO:1). The entire molecule composed of **5**, **10**, **60**, **20** and **30** is referred to here as an affinity labeling reagent or drug affinity label conjugate. To form the target-molecule/entity complex **50**, the entity/reactive functional group bond **60** cleaves and an amine group **70** on the target molecule **40** forms an amide bond **80** via a B<sub>AC</sub>2 mechanism with the entity **10**. The reactive-group/affinity-group molecule **90** is liberated. The specific experimental conditions and product characterization results for this reaction are described in detail in the "Examples " section.

**Please replace the paragraph starting on line 15 of page 24 of the specification with the following amended paragraph:**

In this section, we first describe the construction of S-, O-, and N-linked affinity label libraries: examples 1, 2, and 3 respectively. We then show the results for screening of an S-linked library against HSA at the level of 81 compounds per well (example 4), 9 compounds per well (example 5), and 1 compound per well (example 6). The results of these experiments indicate that -F/YEE (SEQ ID NO:1) is the preferred affinity group for bonding to HSA. Next, results of experiments characterizing the bonding of biotin to HSA using a -F/YEE affinity group (SEQ ID NO:1) are shown. Specifically, example 7 describes methods and materials used for the biological experiments and in examples 8 through 14, we show the results of these experiments.

**Please replace the paragraph starting on line 14 of page 45 of the specification with the following amended paragraph:**

Figures 6a and 6b show optical density measurements from screening of the 9 wells. These results show that molecules of the formula Biotin-S-Ph-C(O)-F-I -Y-X<sub>2</sub>-E-NH<sub>2</sub> show enhanced affinity for HSA. The binding determinant F-I -Y-X<sub>2</sub>-E has SEQ ID NO: 2.

**Please replace the paragraph starting on line 16 of page 48 of the specification with the following amended paragraph:**

**Example 8: Biological Results—Non-specificity of biotin-OPh-CO-NH<sub>2</sub>: in absence of " F/YEE " peptide (SEQ ID NO:1), the biotinylated compound loses specificity**

**Please replace the paragraph starting on line 1 of page 51 of the specification with the following amended paragraph:**

The specificity with which biotin-OPh-CO-F/YEE-NH<sub>2</sub> reacts with a specific lysine residue on HSA was determined by mass spectral analysis of a tryptic digest of LC-biotin conjugated to HSA by LC-biotin-OPh-CO-F/YEE-NH<sub>2</sub> (Figures 15A and 15B). Using LC-biotin-OPh-CO-F/YEE-NH<sub>2</sub> instead of biotin-OPh-CO-F/YEE-NH<sub>2</sub> facilitated a larger mass separation in the mass spectrum and identification of HSA from HSA:LC-biotin. The differences in the two chromatograms are not immediately obvious, however; careful analysis of these chromatograms reveals two new peaks (Figures 15A and 15B, arrows indicate the new peaks at 78 and 85 minutes). Mass spectral analysis of the new peak at 78 min showed a new peptide fragment with a mass of 2887.6 which corresponds to a peptide sequence of <sup>501</sup>EFNAETFNAETFTFHADIBTLSELYSKER<sup>521</sup> (where B = carboxymethyl cysteine) (SEQ ID NO: 3) containing only one lysine residue where LC-biotin is attached, Lys<sub>519</sub>. Mass spectral analysis of the new peak at 85 min showed a new peptide fragment with a mass of 5903.4, corresponding to a peptide sequence of: <sup>476</sup>BBTESLVNRRPBFSALEVDETYVPK<sup>500</sup>EFNAETFNAETFTFHADIBTLSEKER<sup>521</sup> (where B = carboxymethyl cysteine) (SEQ ID NO: 4), which contains two lysine residues

Appln. No. 09/530,298  
Resp to NTC of May 8, 2003  
Docket: 500862000700

(K<sub>500</sub> and K<sub>519</sub>). This mass accounts only for the addition of one LC-biotin and since the fragment at 78 min contains only one Lys residue and LC-biotin is attached to it, and this peptide sequence contains the previous sequence, the only lysine residue in this sequence where biotin could be attached is Lys<sub>519</sub>.

Appln. No. 09/530,298  
Resp to NTC of May 8, 2003  
Docket: 500862000700

**In the Sequence List**

After the abstract, please add the SEQUENCE LISTING that is set out on the enclosed separate sheets.